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DATA EVALUATION REPORT

Study Type: One-year oral (by capsule) toxicity study in dogs

Chemical: Trifluralin (Compound 036352);
Caswell No. 889 **DP Barcode:** D182358
MRID No. 424470-01 **EPA Case No.** 818802
EPA ID No. 036101 **Submission:** S424973

Sponsor: DowElanco
Quad IV
9002 Purdue Rd,
Indianapolis, IN 46268-1189

Testing Facility: Toxicology Research Laboratories
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Citation: Adams, E.R., Bernhard, N.R., and Jordan, W.H. (1992) A chronic toxicity study of trifluralin (Compound 036352) administered orally to beagle dogs for 1 year. Unpublished study by Toxicology Research Laboratories, Lilly Research Laboratories. Study No. D07190. August 6, 1992. Submitted to EPA by Dow Elanco. EPA MRID No. 424470-01.

Conclusion: Groups (Groups 00, 01, 02, & 03) of beagle dogs (4/sex/dose) orally received trifluralin by capsule at doses of 0, 0.75, 2.4, and 40 mg/kg, respectively, for a year.

The results showed that trifluralin at doses of 0.75 and 2.4 mg/kg produce minimal or no toxicity. At 40 mg/kg, the test article produced the following compound-related effects:

1. a decrease in the body weights of female dogs towards the last 6 months of the study. The decrease was approximately 15% relative to the body weight of the controls,
2. a decrease in erythrocyte counts and hemoglobin in male and female dogs,
3. an increase in thrombocyte counts in male and female dogs,
4. an increase in methemoglobin in male and female dogs,
5. an increase in the cholesterol and triglyceride levels in males,

6. an increase in the absolute liver weights and the ratios of liver:body weight and liver:brain weight in males and females, and
7. a decrease in absolute heart weights and the ratio heart:brain weight.

Based upon the results of decrease in body weights, decrease in erythrocytes, increase in methemoglobin, and increase in absolute and relative liver weights, and increase in cholesterol and triglyceride, the LOEL was 40 mg/kg; NOEL, 2.4 mg/kg.

This study for the most part meets the data requirements for a chronic non-rodent toxicity study (Guideline No. 83-1), and it is classified as core minimum.

Methods and Materials

Test Article: Trifluralin (Compound 036352); 2,6-dinitro-N,N-di(n-propyl)- α,α,α -trifluoro-p-toluidine. Lot No. 326EF8. The report does not contain any information on the chemical analysis and the purity of the compound except the potency which is reported to be 99.8%.

Test Animals: Beagle dogs were obtained from Marshall Farms, North Rose, NY. The test animals were acclimated to the laboratory for a minimum of 3 weeks. At the initiation of the study, these dogs were 4 months old and weighing 6.3 ± 0.59 kg for males and 5.3 ± 0.47 kg for females.

Study Design: 16 male (≈ 6.3 kg) and 16 female dogs (≈ 5.37 kg) were selected based on their better health status among the dogs received. These dogs were randomly divided into the test groups shown in Table 1.

Table 1+. Dose Levels and Number of Dogs in Each Dose Group

| <u>Group</u> | <u>Dose levels mg/kg/day</u> | <u>No. of dogs/dose group</u> | |
|--------------|----------------------------------|-------------------------------|----------------|
| | | <u>Males</u> | <u>Females</u> |
| 00 | 0 | 4 | 4 |
| 01 | 0.75 | 4 | 4 |
| 02 | 2.4 | 4 | 4 |
| 03 | 40 | 4 | 4 |

+: Data tabulated from page 17 of the report (MRID # 424470-01).

Administration: The selected test animals were housed

individually and received the test article in capsule which was prepared by placing a weighed amount of the test article in gelatin capsules. Individual doses were adjusted weekly using the most recent body weight value for each animal. The control dogs received empty gelatin capsules. The test dogs were housed individually and fed approximately 300 gm of Purina Certified Canine Diet No. 5007 every morning after dosing. Water was available ad lib.

Clinical observation: The test animals were observed several times daily during the week days and once daily during weekends or holidays for signs of toxicity and mortality. The daily animal observations included posture, behavior, tremors, emesis, abnormal stool, color of mucous membranes, skin, and hair coat, and the presence of any abnormal growth.

Body weights: The body weights of each dog were determined during pretest and weekly during the study.

Food consumption: Food consumptions were estimated at pretest and daily during the study.

Ophthalmology: Ophthalmoscopic examinations were conducted on all dogs by a veterinary ophthalmologist once at pretest period, at 6 months, and at the termination of the study. The initial examinations consisted of evaluating the pupillary light reflex under reduced illumination. The adnexa, cornea, sclera, anterior chamber, iris, lens, and fundus were evaluated under the influence of one or two drops of 1% tropicamide (Mydriacyl[®]).

Clinical pathology: Clinical pathology was conducted on each dog prior to the initiation of the study and at months 1, 3, 6, and 12 (termination) of the study.

Hematology: The following hematological parameters were measured:

| | |
|-------------------------|-------------------------------------|
| leukocyte counts | erythrocyte counts |
| hemoglobin | hematocrit |
| Mean corp. volume (MCV) | mean corp. hemoglobin concentration |
| mean corp. hemoglobin | platelet counts |
| reticulocyte counts | differential leukocyte counts |
| erythrocyte morphology | thrombocyte count |
| activated partial | prothrombin time |
| thromboplastin time | bone marrow smears |

Clinical chemistry: The following parameters were examined:

| | |
|------------------------------|----------------------------|
| sodium | potassium |
| chloride | calcium |
| inorganic phosphorus | alkaline phosphatase |
| total bilirubin | direct bilirubin |
| aspartate aminotransferase | alanine aminotransferase |
| (AST) | (ALT) |
| creatine phosphokinase (CPK) | urea nitrogen |
| creatinine | total protein |
| albumin | globulin |
| albumin/globulin (A/G) | cholesterol |
| glucose | lactic dehydrogenase (LDH) |
| leucine aminopeptidase (LAP) | triglycerides |
| methemoglobin | gamma glutamyl transferase |

Urinalysis: The urine samples were also collected prior to the initiation of the study and at 1, 3, 6, and termination of the study. The following parameters were analyzed for urine:

| | |
|--------------------|----------------------|
| Color & appearance | volume |
| specific gravity | microscopic elements |
| pH | protein |
| glucose | ketones |
| bilirubin | occult blood |
| urobilinogen | |

Gross and Histopathology

All test animals received a postmortem examination. All macroscopic abnormalities were recorded.

Organ weights: Representative tissue samples were collected from each animal and fixed in phosphate-buffered neutral formalin where appropriate. The following organs were weighed:

| | |
|-------------------|---------------------|
| adrenals | liver |
| brain (with stem) | ovaries |
| heart | kidneys |
| testes | thyroid/parathyroid |

Relative organ weights (organ weight/body weight or brain weight) were calculated.

Histological examinations were conducted on the tissue samples collected at the end of the study. The following tissues were collected for microscopic examinations:

| | |
|--------------------|--------------------------------|
| adrenals | lymph nodes (mesenteric & |
| aorta | cervical) |
| bone | bone marrows (sternum & femur) |
| bone marrow smears | mammary glands |

brain
 eye
 esophagus
 duodenum
 ileum
 colon
 prostate
 sciatic nerve
 ovaries
 skeletal muscle
 spinal cord
 thymus
 heart
 kidneys
 urinary bladder
 lungs
 gross lesions

pancreas
 pituitary
 stomach
 jejunum
 cecum
 rectum
 salivary glands
 gall bladder
 testis with epididymis
 skin
 spleen
 thyroid/parathyroid
 trachea
 liver
 uterus
 vagina
 cervix

Statistical analysis: Dunnett's statistical method was used in the analysis of the differences between controls and the treated group means for parameters whose data were distributed normally. Bartlett's method described by Steel and Torrie was used in testing the homogeneity of variances. Statistical significance is represented by $p < 0.05$ for the Dunnett's test and $p < 0.001$ for the Bartlett's test.

A quality assurance statement and Good Laboratory Practice statement were signed and included in the report.

A statement of no claim of confidentiality and a statement that this study did not meet the criteria for flagging of studies for potential adverse effects were also submitted in the report.

RESULTS

1. Test diet analysis

Test article purity and stability : The purity of the chemical was assayed against a standard and expressed as the potency of the chemical, and it was reported to be 99.8%. The stability of the test article was measured by the persistence of its potency over a period of several months. The test results showed that the test article was stable at various assay periods (May 16, 1991 or October 11, 1991).

2. Mortality: All dogs survived to the end of the study.

3. Clinical observations: Although soft stools were reported for both male and female controls and treated dogs, compound-related clinical signs were not observed.

4. Body weights: The report did not contain the values of the mean body weights for the entire period of the study. It presented the data on the means of weekly measurement for each dose group, and these values were plotted in Figures 1 & 2. Based upon the weekly results, this reviewer calculated the average body weights for the entire test period by using the values of every 5 weeks. The calculated values are presented in Table 2. In male dogs, the body weights of Groups 01 & 02 were slightly more than that of the controls, while that of Group 03 was comparable to those of the controls (Fig 1 & Table 2).

In female dogs, there was a slight drop in body weights of Groups 01 and 02. More body weight decrease was found in Group 03 (Table 2 and Figure 2). Toward the last six months of the study, the body weight decrease in Group 03 was approximately 15%, while the decrease in the mean body weight throughout the study was approximately 10%.

The report also presents mean body weight changes as percent of the initial weights for various groups of animals, the results indicated that the Group 03 females showed a substantial lag in this change (Control 103.87%; Group 03, 80.72%) at the termination of the study.

Table 2+. Mean Body Weights of Trifluralin Treated Male and Female Dogs (by oral capsule)

| Group | Dose levels mg/kg/day | Mean Body Weights in 52 Weeks (kg) | |
|-------|--------------------------|------------------------------------|-------------|
| | | Males | Females |
| 00 | 0 | 10.59 ± 1.40 | 9.57 ± 1.55 |
| 01 | 0.75 | 11.00 ± 1.50 | 9.50 ± 1.32 |
| 02 | 2.4 | 11.30 ± 1.61 | 9.23 ± 1.29 |
| 03 | 40 | 10.44 ± 1.42 | 8.62 ± 0.88 |

+ : Data calculated from Tables 3.1 & 3.2 of the report (MRID No. 424470-01).

5. Food and compound consumptions: The report stated that food consumption was estimated visually each day and any changes in appetite were noted.
6. Ophthalmological examinations: No compound-related ophthalmological changes were found.
7. Hematology: There were slight changes (sometimes statistically significant) in the hematological parameters which were treatment-related. The changes were confined to the male and female dogs of Groups 03. The changes included a decrease in erythrocyte counts, a decrease in hemoglobin, and an increase

in mean corpuscular volume (Tables 3 & 4).

An increase in the thrombocyte counts was seen in both male and female dogs of Group 03; the increase in males was significantly different from those of the controls at 3 and 6 months and at the termination of the study, while those in the females did not show a statistical significance (Table 5). Other slight changes were also seen, but they were sporadic and could not be considered as treatment-related effects.

8. Clinical chemistry: In general the methemoglobin levels dropped as time progressed in the study. However, there was an increase in methemoglobin level in Group 03 male and female dogs in comparison to that of the controls at various examination periods. The increase showed a significant difference from the controls at 6 month for males and at 6 and 12 months for females, and sometimes the increase approached 4 (male) or 7 (female) times the controls (Table 6). This observation was consistent with the findings of a decrease in hemoglobin level in this dose group (Tables 3 & 4).

An increase in the cholesterol level was seen in Group 03 males at 1, 3, 6, and 12 months examination periods, and the increase was statistically significant ($p < 0.05$) at 6 and 12 months examination intervals (Table 7). Triglyceride level was also increased in Group 03 males, and it was statistically significant at the 6 month examination interval (Table 7). A slight increase in cholesterol level was seen in Group 03 females, but the increase was not statistically significant.

The alkaline phosphatase (ALP) activity of the controls, Group 01, and Group 02 males and females showed a time-related decrease, which was not unexpected for young animals whose ALP level often decrease as they age (Tables 8 & 9). However, Both males and females of Group 03 dogs did not showed a similar decrease at 6 and 12 months examination intervals, and, based upon the patterns of the controls and the two lower dose groups, one would have drawn the conclusion that the ALP levels of the high dose males and females were increased relative to those of the controls. In examining the individual data, the apparently increase was due to one male and one female dog which had unusually high ALP levels. Although the data showed a consistency between sexes, the toxicological significance of the changes in the ALP level in Group 03 animals was difficult to determine because of the small number of the test samples.

There was also a decrease in the alanine aminotransferase (ALT) activities in Group 03 males and in Groups 02 & 03 females at 12 month examination period relative to those of the controls, but these values were comparable to the

corresponding pretest values (Tables 8 & 9) . A slight decrease in aspartate aminotransferase level was also seen in Group 03 males and females at the 12 month examination period. The toxicological significance of the decrease in ALT and AST in the high dose dogs could not be determined since the histopathological data did not show any changes, and, in comparison to the corresponding values of the pretest period, the changes were none or slight at best.

9. Urinalysis: The means of the urinalysis parameters were comparable between the treated groups and the controls.
10. Macroscopic pathology: Gross pathology did not show any compound-related effects except yellow decoloration of fat in one male and one female of Group 03 dogs.
11. Organ weights: The mean absolute and relative (liver/body and liver/ brain) liver weights in Group 03 males and females were increased in comparison to those of the controls (Tables 10, 11, & 12). There was a decrease in absolute heart weight and the relative heart weight (heart/brain) in high dose females. The heart/brain weight was also decreased in Group 02 females (Tables 10 & 12).
12. Histopathology: Compound-related histopathological findings were not reported.

DISCUSSION

Groups of beagle dogs (4/sex/dose) orally received trifluralin by capsule at doses of 0, 0.75, 2.4, and 40 mg/kg for a year. The doses were selected based upon the results of 4 previous studies in dogs with trifluralin; three studies were conducted with oral capsule administration and one with dietary administration. The low dose (0.75 mg/kg) was the value of the NOEL of the most current of the previous 4 studies and the high dose (40 mg/kg) was selected to ensure some kinds of definitive compound-related effects because in the previous 4 studies the highest dose (25 mg/kg) produced "either no toxicity or minimal evidence of toxicity".

The results of the present study showed that trifluralin at doses of 0.75 and 2.4 mg/kg produced minimal or no toxicity. At 40 mg/kg, the test article produced a decrease in the body weights of female dogs towards the last 6 month of the study. The decrease was approximately 15% relative to the body weight of the controls. No food consumption data was presented to substantiate whether the decrease in body weights was due to the effect of compound or to a decrease in food intake. In the absence of the food consumption data, the decrease in body weights is assumed to be compound-related effect.

Hematology data showed a decrease in erythrocyte counts and hemoglobin in 40 mg/kg male and female dogs. An increase in thrombocyte counts was also seen in 40 mg/kg male and female dogs.

Clinical chemistry data indicated an increase in methemoglobin in 40 mg/kg male and female dogs. An increase in the cholesterol and triglyceride levels was seen in 40 mg/kg males.

The absolute liver weights and the ratios of liver:body weight and liver:brain weight were increased in 40 mg/kg males and females. The absolute heart weight and ratio of heart/brain weight were decreased in 40 mg/kg females. No compound-related histopathological changes were seen in any dosed groups.

Based upon the results of decrease in body weights, decrease in erythrocytes, increase in methemoglobin, increase in absolute and relative liver weights, and increase in cholesterol and triglyceride, the LOEL was 40 mg/kg; NOEL, 2.4 mg/kg.

This study for the most part meets the data requirements for a chronic non-rodent toxicity study (Guideline No. 83-1), but it lacks the data on food consumption to determine whether the decrease in body weights seen in 40 mg/kg males was due to the compound or a decrease in food intake. However, the missing information did not present any major difficulty in interpreting the findings. Also the dogs were feed with a measured amount of food each day (300 gm). The study is classified as core minimum.

It should be noted that this study was conducted to mainly satisfy California EPA's request. An acceptable 1 year feeding-study in dogs with trifluralin is available (EPA Accession No. 259001).

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Pages 10 through 22 are not included.

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